

## Two new species of the *griseifrons* species-group of *Simulium* (*Simulium*) (Diptera: Simuliidae) in northern Thailand

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**Abstract:** Two new species of the *griseifrons* species-group of the subgenus *Simulium* (*Simulium*), i.e., *S. (S.) phayaoense* sp. nov. and *S. (S.) pukaengense* sp. nov., are described on the basis of the observation of females, males, pupae and mature larvae collected from northern Thailand. Morphological characteristics differing from their related known species are noted.

**Key words:** black fly, Simuliidae, *Simulium*, Thailand, new species, *griseifrons* species-group

In Thailand, the *griseifrons* species-group of the subgenus *Simulium* (*Simulium*) is represented by 10 species (Takaoka and Chochote, 2004a, b). During recent surveys on the immature stages of black flies in northern Thailand, we collected two new species, both of which are assigned to this species-group by having the simple female claws, the male style with a prominent basal protuberance, the male ventral plate without toothed margins, the pupal gill with six filaments on each side, and the bullet-shaped larval postgenal cleft.

These two new species are described on the basis of adults (reared from pupae), pupae and mature larvae.

The terms for morphological features used here follow those of Takaoka (2003). Holotype and most paratype specimens of the new species are deposited at the Department of Infectious Disease Control, Faculty of Medicine, Oita University, Oita, Japan.

### *Simulium* (*Simulium*) *phayaoense* sp. nov.

**DESCRIPTION. Female.** Body length 3.5 mm. **Head.** Narrower than thorax. Frons blackish-brown, shiny, with several dark stout hairs along lateral margins; frontal ratio 1.2 : 1.0 : 1.2; frons-head ratio 1.0 : 4.2. Fronto-ocular area (Fig. 1A) well developed, directed laterally and somewhat upwardly, and rounded apically. Clypeus blackish-brown, whitish-grey pruinose, shiny at certain angle of light, and moderately covered with dark stout hairs except upper 2/5 narrowly bare medially. Labrum about 0.63 times as long as clypeus. Antenna composed of 2+9 segments, whitish-yellow on scape and pedicel, orange-yellow though gradually darkened to light brown apically on flagellar segments 1–7, and medium brown on flagellar segments 8 and 9; 1st flagellar segment about 1.5 times as long as 2nd flagellar segment. Maxillary palp with 5 segments, dark brown to blackish-brown, proportional lengths of 3rd, 4th, and 5th segments 1.0 : 1.0 : 2.3; 3rd segment (Fig. 1B) of normal size, with sensory vesicle oblong, 0.41 times as long as 3rd segment. Maxillary lacinia with 11

or 12 inner and 14 or 15 outer teeth. Mandible with 24 inner and 10 or 11 outer teeth. Cibarium (Fig. 1C) with very short blunt medial projection directed dorsally at its posterodorsal margin, with about 20 minute processes on its surface, and also with 13 minute processes near base of this medial projection.

**Thorax.** Scutum brownish-black (except anterolateral calli light brown), shiny, grey pruinose, with scutal patterns formed by pruinose and non-pruinose portions differing by the angles of light: i.e., when illuminated anterodorsally and viewed dorsally, 1 medial narrow, 2 submedial wide (nearly twice or a little less as wide as medial vitta), and 2 sublateral rather wide (somewhat wider than submedial ones) longitudinal dark non-pruinose vittae, all connected to large dark non-pruinose portion on prescutellar area; color pattern reversed when illuminated dorsally and viewed posteriorly; scutum densely covered with yellow recumbent short hairs interspersed with dark long upright hairs on prescutellar area. Scutellum light to medium brown, with dark long hairs as well as yellow short hairs. Postnotum brownish-black, shiny, and bare. Pleural membrane bare. Katepisternum longer than deep, shiny, and bare. **Legs.** Foreleg: coxa whitish-yellow; trochanter whitish-yellow though somewhat darkened apically; femur light to medium brown except inner surface yellowish to light brown; tibia white except apical 1/4 or 1/5 brownish-black, with a large white sheen on outer surface when illuminated; tarsus brownish-black, with somewhat thick dorsal hair crest; basitarsus greatly dilated, 4.7 times as long as its greatest width. Midleg: coxa brownish-black; trochanter light brown except base pale yellow; femur medium to dark brown; tibia white except apical 1/5 (or a little more) medium brown; tarsus medium to dark brown except basal 3/4 of basitarsus and base of 2nd segment yellowish-white. Hind leg: coxa dark brown; trochanter whitish yellow; femur medium brown except basal 1/5 whitish-yellow and apical cap dark brown; tibia (Fig. 1D) medium brown to brownish-black with basal 2/5 white to yellowish-white though basal 3/4 white or yellowish-white on posterior surface; tarsus brownish-black except a little more than basal 1/2 of basitarsus yellowish-white and basal 1/2 of 2nd segment dark yellow; basitarsus (Fig. 1E) nearly parallel-sided, about 5.3 times as long as wide,

and 0.76 and 0.68 times as wide as greatest widths of hind tibia and femur, respectively; calcipala (Fig. 1E) moderately developed, 0.7 times as long as wide; pedisulcus (Fig. 1E) well developed at basal 1/3 of 2nd tarsal segment. All tarsal claws simple. **Wing.** Length 2.5 mm. Costa with dark spinules and hairs; subcosta haired except apical 1/4 bare; basal section of radial vein haired except basal 1/5 or 1/6 bare; R<sub>1</sub> with dark spinules and hairs; R<sub>2</sub> with hairs; hair tuft on stem vein dark brown. **Abdomen.** Basal scale dark brown, with fringe of dark brown ones. Dorsal surface of abdomen medium brown to brownish-black except basal 1/2 of 2nd segment whitish-yellow, with dark short hairs; tergite 2 white pruinose and shiny at certain angles of light, tergites 3–5 small, dull, and tergites 6–9 wide and shiny. Ventral surface of abdomen greyish to light brown except segment 8 brownish-black; ventral surface of segment 7 without sternal plate. **Genitalia.** Sternite 8 (Fig. 1F) strongly sclerotized, with 11 or 12 long and medium-long stout hairs as well as 4 or 5 short fine hairs on each lateral surface. Ovipositor valves in ventral view (Fig. 1F) round, flap-like, or short tongue-like in ventrolateral view (Fig. 1G), curved ventrally with inner margins completely turned ventrally, membranous, covered with 11 or 12 short hairs as well as numerous microsetae; ovipositor valves each with a long narrow transparent projection directed dorsomedially from dorsal surface. Genital fork (Fig. 1H) of inverted-Y form, with narrow, well sclerotized stem and narrow, weakly sclerotized arms; each arm with distinct well-sclerotized triangular projection directed anteriorly. Paraproct in ventral view (Fig. 1I) somewhat wide anteriorly (nearly as wide as its length), with distinct concavity on anterolateral surface, and with 12 or 13 sensilla on unpigmented anterior surface; paraproct in lateral view (Fig. 1J) about 1.2 times as wide as its length, moderately protruding ventrally beyond cercus, with numerous stout hairs on lateral and ventral surfaces. Cercus in lateral view (Fig. 1J) short (about 0.4 times as long as wide), rounded posteriorly, and with numerous stout hairs. Spermatheca (Fig. 1K) globular or somewhat ovoid, well sclerotized (except portion of junction to duct unsclerotized), polygonal surface patterns markedly defined near junction but faintly on rest of surface, and with internal setae; accessory ducts subequal in thickness to major one.

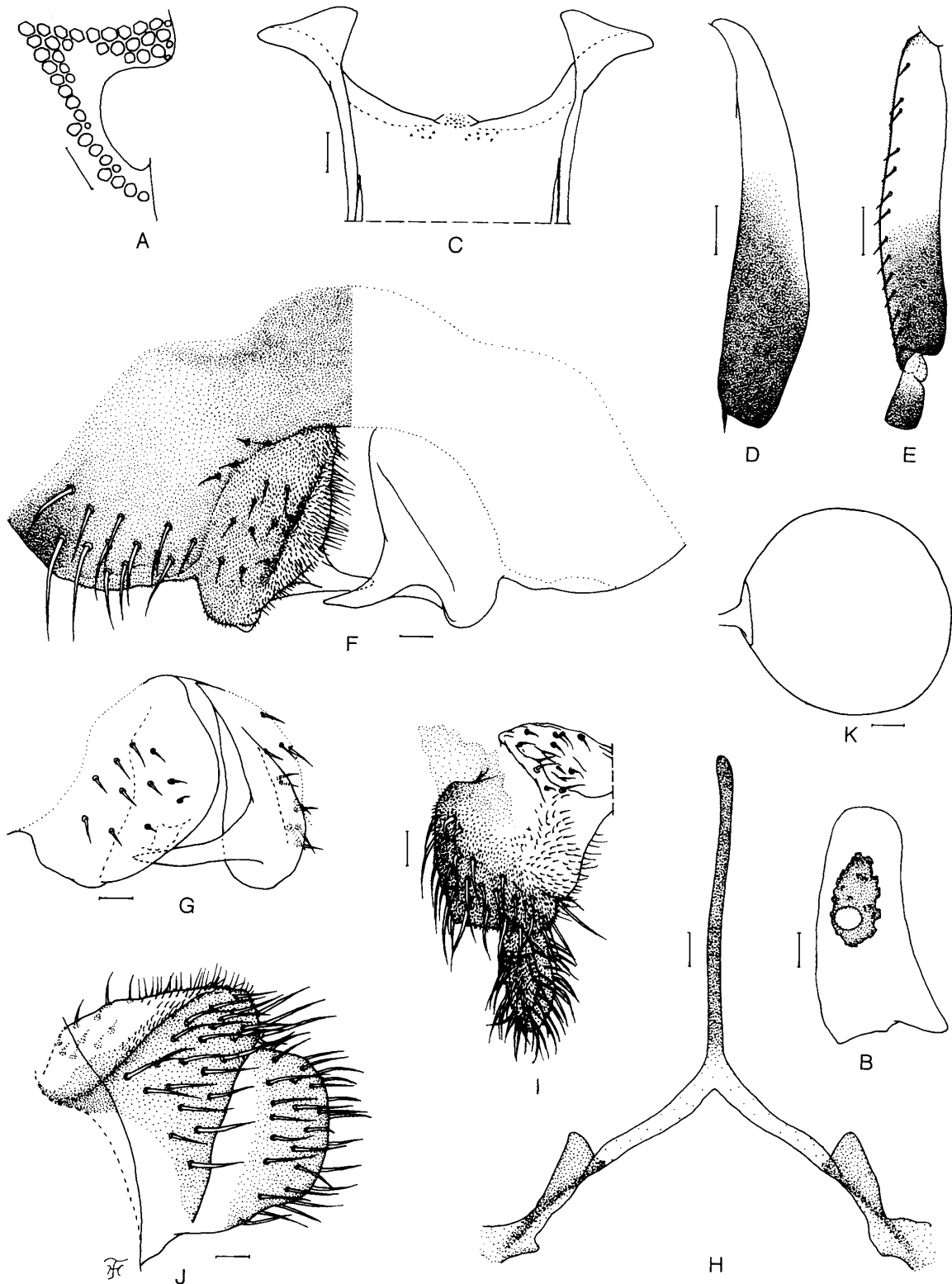


Fig. 1. Female of *Simulium* (*Simulium*) *phayaoense* sp. nov. A, fronto-ocular area (right side); B, 3rd segment of maxillary palp with sensory vesicle (right side, front view); C, cibarium; D, hind tibia (left side, outer view); E, hind basitarsus and 2nd tarsal segment (left side, outer view); F, 8th sternite and ovipositor valves (ventral view); G, ovipositor valves (ventrolateral view); H, genital fork; I and J, paraprocts and cerci (right side; I, ventral view; J, outer view); K, spermatheca. Scale bars. 0.1 mm for D and E; 0.05 mm for A; 0.02 mm for B, C, and F-K.

**Male.** Body length 2.6–3.3 mm. **Head.** Nearly as wide as thorax. Upper eye with large facets in 17 vertical columns and in 18 horizontal rows. Face and clypeus black, thickly white pruinose (silvery iridescent when illuminated at certain angle of light), sparsely covered with dark brown long hairs. Antenna composed of 2 + 9 segments, yellow or dark yellow except 2–5 apical flagellar segments medium brown; 1st flagellar segment elongate, 1.8 times as long as wide, and 1.9 times as long as 2nd flagellar segment. Maxillary palp with 5 segments, medium to dark brown, proportional lengths of 3rd, 4th, and 5th segments 1.0 : 1.2 : 2.9; sensory vesicle (Fig. 2A) small, oblong, 0.23–0.28 times as long as 3rd segment, with small opening apically. **Thorax.** Scutum black, densely covered with golden-yellow recumbent short hairs interspersed with brown similar hairs anteriorly, and with dark brown long upright hairs on prescutellar area; scutum with whitish-grey pruinose spots, i.e., anterolateral pair of large rectangular spots on shoulders which connect widely to posterior large spot on prescutellar area through spots along lateral margins; all these spots usually not so distinct due to thick covering of golden-yellow short hairs, but silvery iridescent when illuminated at certain angle of light. Scutellum dark brown to brownish-black, with golden-yellow short hairs as well as dark brown long upright hairs. Postnotum brownish-black, white pruinose, and bare. Pleural membrane bare. Katepisternum longer than deep, and bare. **Legs.** Foreleg: coxa yellowish-white; trochanter light brown except inner surface white medially; femur light brown except basal 1/2 (or a little more) of inner surface yellowish-white; tibia medium to dark brown with outer surface of basal 3/4 (or a little more) whitish, and sheeny when illuminated; tarsus dark brown to brownish-black, with moderate dorsal hair crest; basitarsus somewhat dilated, 6.0 times as long as its greatest width. Midleg: coxa brownish-black; trochanter dark brown except base narrowly yellowish; femur medium brown; tibia white to yellow except apical 1/5 medium brown; tarsus medium brown except basal 2/3 yellow. Hind leg: coxa dark brown to brownish-black; trochanter yellowish-white; femur and tibia brownish-black except base yellowish-white; tarsus dark brown to brownish-black except basal 1/2 of basitarsus and a little less than basal 1/2 yellowish-white; basitarsus (Fig. 2B)

enlarged, spindle-shaped (very slightly widened from base to apical 1/3, then gradually narrowed toward apex), 4.8 times as long as its greatest width, 0.76 and 0.73 times as wide as greatest widths of hind tibia and femur, respectively; calcipala well developed, nearly as long as its width at base; pedisulcus well developed. **Wing.** Length 2.1 mm; Costa with dark spinules and hairs; subcosta bare; basal section of radial vein bare;  $R_1$  with dark spinules and hairs;  $R_2$  with hairs; hair tuft on stem vein dark brown; basal cell absent. **Abdomen.** Basal scale brownish-black, with fringe of dark brown long hairs. Dorsal surface of abdominal segments brownish-black except a little more than basal 1/3 of segment 2 whitish-yellow, with dark short hairs; tergites 2, 6 and 7 each with a pair of silvery iridescent dorsolateral spots, those on tergite 2 connected broadly to each other medially. **Genitalia.** Coxite in ventral view (Fig. 2C) subquadrate, somewhat longer than wide, with stout hairs on posterior 1/2 of ventral surface. Style in ventral view (Fig. 2C) much longer than coxite, gently curved inwards, moderately covered with stout hairs on ventral and lateral surfaces, and with single terminal spine; style in ventrolateral view (Fig. 2D) nearly parallel-sided or very slightly narrowed from base to apex; style in medial view (Fig. 2E) with distinct basal protuberance directed dorsomedially, with several small cone-shaped spines at and near apex. Ventral plate in ventral view (Fig. 2C) with its body broad, lamellate, about 0.4 times as long as its greatest width, with its posterolateral corners rounded, moderately covered with microsetae except on both lateral areas, and basal arms strongly sclerotized and directed forward, nearly parallel-sided or directed forward and curved inward, then somewhat converged; ventral plate in end view (Fig. 2F) with low median process directed ventrally, moderately covered with microsetae centrally, and with ventral margin on each side appearing to be undulated; ventral plate in lateral view (Fig. 2G) with low posterior process directed ventrally, and with posterodorsal margin appearing to be weakly lacerated. Paramere (Fig. 2H) large basally, with numerous short to long hooks. Median sclerite in posterior view (Fig. 2I) broad, slightly widened toward apex, with round apex, moderately sclerotized and brown. Aedeagal membrane densely setose; dorsal plate in ventral view (Fig. 2J) moderately

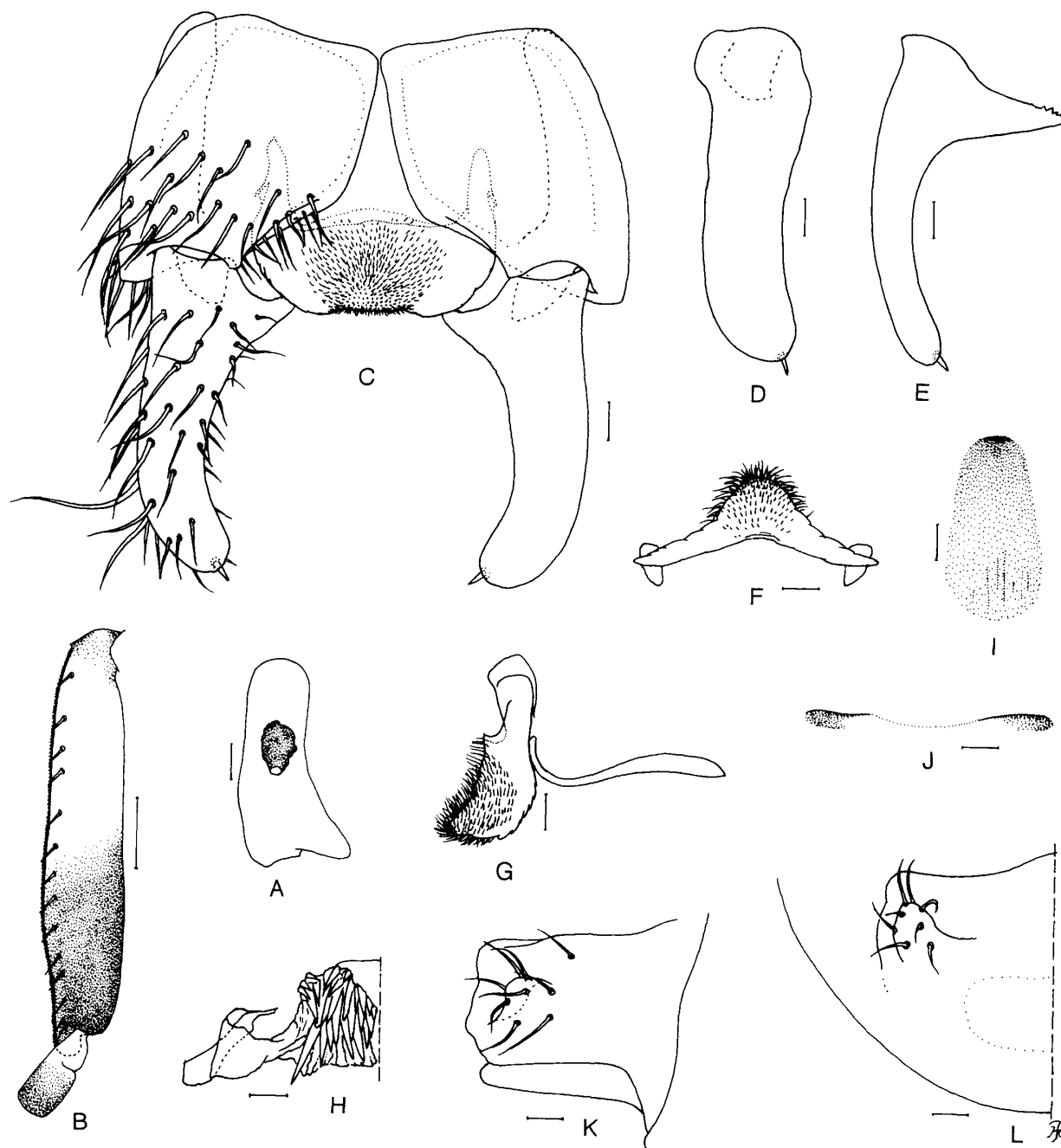


Fig. 2. Male of *Simulium* (*Simulium*) *phayaoense* sp. nov. A, 3rd segment of maxillary palp with sensory vesicle (right side, front view); B, basitarsus and second tarsal segment of hind leg showing calcipala and pedisulcus (left side, outer view); C, coxites, styles and ventral plate *in situ* (ventral view); D, style (right side, ventrolateral view); E, style showing basal protuberance (right side, medial view); F, ventral plate (end view); G, ventral plate and median sclerite (lateral view); H, paramere (right side, end view); I, median sclerite (posterior view); J, dorsal plate (ventral view); K and L, 10th abdominal segments and cerci (K, left side, lateral view; L, right side, end view). Scale bars. 0.1 mm for B; 0.02 mm for A, and C-L.

sclerotized except median portion widely unsclerotized. Abdominal segment 10 (Fig. 2K, L) with or without 1 distinct hair on each lateral surface. Cercus (Fig. 2K, L) small, short, rounded, with 8–13 hairs.

**Pupa.** Body length ca. 3.6 mm. **Head.** Integu-

ment light yellowish-brown, densely and elaborately covered with small round tubercles; antennal sheath densely covered with small round tubercles; face with a pair of long trichome with 6–8 slender branches (Fig. 3A), frons with 2 pairs of long trichomes each with 2–4 slender

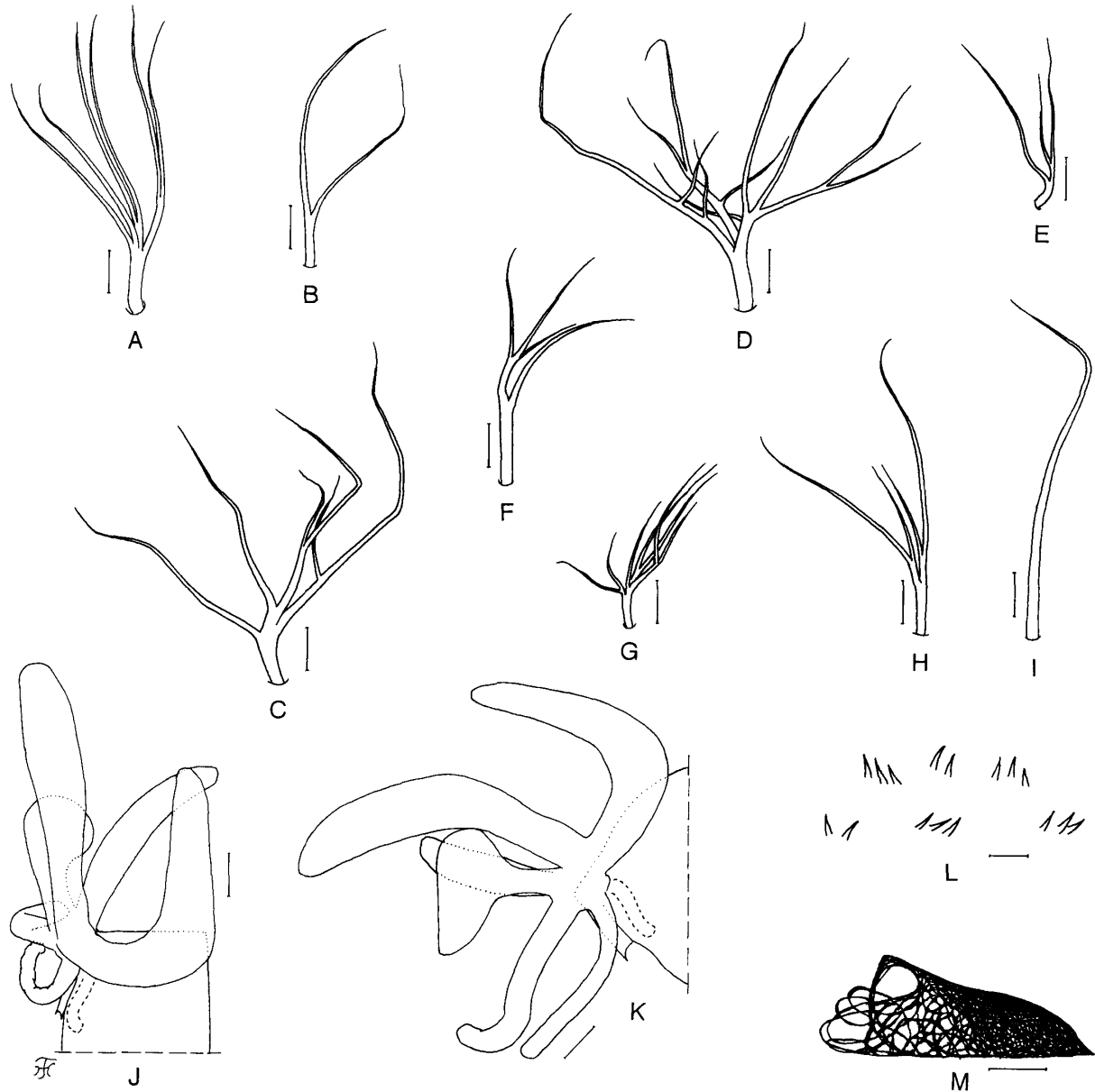


Fig. 3. Pupa of *Simulium* (*Simulium*) *phayaoense* sp. nov. A, facial trichome; B, frontal trichome; C-I, thoracic trichomes (C, anterodorsal; D and E, anterolateral; F, posterolateral; G-I, ventrolateral); J and K, gill filaments (left side; J, dorsal view; K, outer view); L, minute spinous processes on gill filaments; M, cocoon (lateral view). Scale bars. 1.0 mm for M; 0.2 mm for J and K; 0.02 mm for A-I; 0.01 mm for L.

branches (Fig. 3B). **Thorax.** Integument light yellowish-brown, densely and elaborately covered with small round tubercles; thorax on each side with 3 very long trichomes each with 6 or 7 slender branches (Fig. 3C) antero-dorsally, 1 very long trichome with 8-11 slender branches (Fig. 3D) and 1 medium-long trichome with 3 or 4 slender branches (Fig. 3E) anterolaterally, 1 medium-long trichome with 3 or 4 branches (Fig. 3F) posterolaterally, and 1 medium-long trichome with 6 or 7 slender trichomes (Fig. 3G), 1 long trichome with 2-4 branches (Fig. 3H), and 1 simple very long

trichome (Fig. 3I) ventrolaterally. Gill (Fig. 3J, K) with 6 short inflated tubular filaments, almost sessile, widely divergent; dorsalmost filament directed upward and slightly inward, then curved forward; 2nd filament from dorsal directed forward and somewhat curved downward; 3rd filament from dorsal directed forward and abruptly curved downward; 4th filament from dorsal lying horizontally in parallel to 3rd filament, directed forward and inward; 5th filament from dorsal directed forward and downward, then curved inward; 6th filament from dorsal directed downward, then

curved forward and slightly inward; all filaments light brown, without annular ridges and furrows, sparsely covered with minute setae (Fig. 3L), and densely covered with minute tubercles. **Abdomen.** Dorsally, segment 1 well sclerotized, light yellowish-brown, almost bare, and with 1 simple or bifid medium-long slender seta on each side; segment 2 transparent, with 1 simple or bifid medium-long slender seta and 4 short stout spines on each side; segments 3 and 4 transparent, each with 4 hooks and 1 short stout spine on each side; segment 5 transparent and bare; segments 6 and 7 each with a transverse row of comb-like groups of minute spines near anterior margin on each side; segment 8 transparent, with a transverse row of distinct spine-combs as well as comb-like groups of minute spines near anterior margin on each side; segment 9 weakly sclerotized, light yellowish brown, very sparsely covered with small round tubercles posteriorly, with a transverse row of distinct spine-combs (though usually smaller in size than those on segment 8) as well as comb-like groups of minute spines near anterior margin on each side; terminal hooks absent. Ventrally, all segments nearly transparent except segment 9 yellow to light yellowish-brown; segments 3–8 each with comb-like groups of minute spines; segment 4 with 1 slender simple hooklet (much smaller than those on segment 5) and a few simple slender setae on each side; segment 5 with a pair of bifid inner and simple or bifid outer hooks submedially and a few simple slender setae on each side; segments 6 and 7 each with a pair of bifid inner and simple outer hooks and a few simple (or bifid) slender setae on each side. **Cocoon** (Fig. 3M). Shoe-shaped, weakly connected anteroventrally forming low to medium-high anteroventral neck, roughly woven with numerous perforations of various sizes, including large dorso-lateral windows, not extending ventrolaterally; individual threads visible; 4.5–5.0 mm long by 1.3–1.8 mm wide.

**Mature larva.** Body length 5.8–6.8 mm. Body color mostly greyish green though brownish to some extent posteriorly, or mostly brownish though greyish green to some extent anteriorly. Cephalic apotome (Fig. 4A) white or yellowish-white on anterior 1/2, dark yellow to yellowish-brown on posterior 1/2 except narrow transverse portion just before posterior margin much darkened; head spots distinctly

darkened, well defined except posterior one of posterolateral spots on each side indistinct; posterior one of mediolongitudinal spots connected posteriorly to dark transverse band just before posterior margin. Lateral surface of head capsule yellow except dorsal and posterior to eye-spot region darkened, with small spot ventral to eye-spot distinctly darkened, but 2 large and 2 small spots near posterior margin all merged into surrounding dark area. Ventral surface of head capsule (Fig. 4B) yellow to dark yellow with broad darkened area along posterior margin on each side of postgenal cleft, and often somewhat darkened broadly between anterior margin of postgenal bridge and posterior margin of hypostomium; horizontal spots on each side of postgenal cleft very dark, often merged into surrounding dark area. Cervical sclerites (Fig. 4A) composed of 1 narrow stick-like and 1 elliptical pieces usually fused to each other on each side, weakly or not fused to occiput, moderately separated medially from each other. Antenna composed of 3 segments and apical sensillum, longer than stem of labral fan; length ratio of segments (from base to tip) 1.0 : 1.2 : 0.5. Labral fan with 40–46 main rays. Mandible (Fig. 4C) with mandibular serrations composed of 2 teeth (1 large, 1 small), without supernumerary serrations; large mandibular tooth at obtuse angle to mandible on apical side; 1st tooth of comb-teeth longer than 2nd one which is subequal to, or a little longer than, 3rd one. Hypostomium (Fig. 4D) with 9 apical teeth, of which each lateral tooth slightly longer than median tooth; intermediate teeth on each side much shorter than median tooth; lateral serrations moderately developed on anterior 1/2; 4 or 5 hypostomal bristles diverging posteriorly from lateral border on each side. Postgenal cleft (Fig. 4B) medium-sized, nearly parallel-sided on basal 1/2 or more, then narrowed apically, nearly as long as or a little longer than its greatest width, and 2.4–2.8 times as long as postgenal bridge. Pharate pupal gill with 6 inflated filaments, of which 4 filaments and apical portion of 1 filament are visible in outer view (Fig. 4E). Thoracic cuticle bare. Abdominal cuticle bare except dorsolateral areas on last segment densely covered with minute discolored setae. Rectal scales present. Rectal organ compound, each of 3 lobes with 11–15 finger-like secondary lobules. Anal sclerite X-shaped, with short broad anterior arms 0.6 times as long as posterior ones; basal

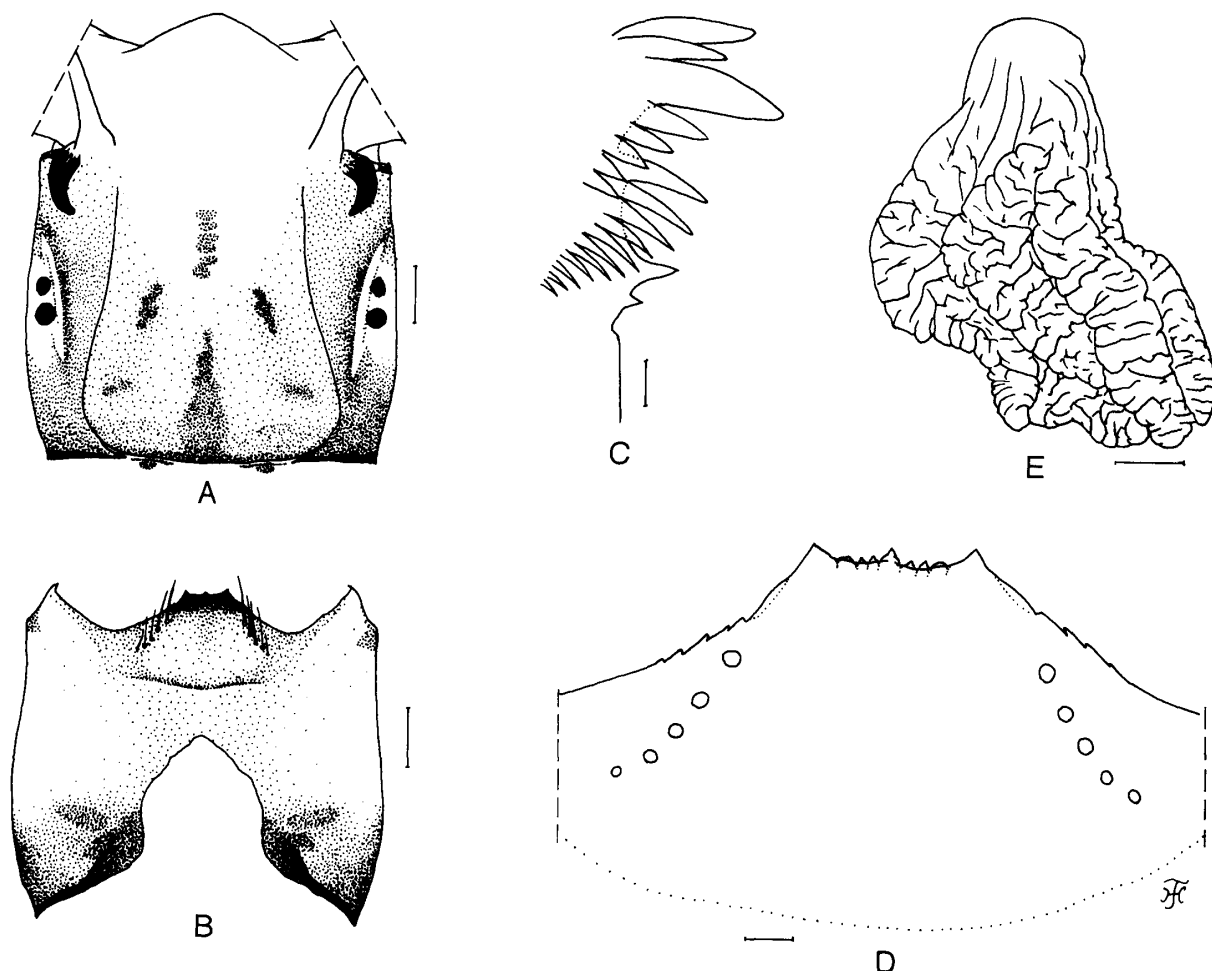


Fig. 4. Mature larva of *Simulium (Simulium) phayaoense* sp. nov. A, dorsal surface of head capsule showing head spots on clypeus; B, ventral surface of head capsule showing postgenal cleft; C, apical portion of mandible; D, hypostomium; E, pharate pupal gill (left side, outer view). Scale bars. 0.1 mm for A, B and E; 0.02 mm for D; 0.01 mm for C.

juncture area with deep narrow incision opening posteriorly; 4–6 sensilla on basal juncture area and 6 or 7 sensilla posterior to posterior arms. Posterior circlet with 84–98 rows of hooklets with up to 17 hooklets per row. Ventral papillae absent.

**TYPE SPECIMENS.** Holotype female with its associated pupal exuvia and cocoon, collected from Champa Thong Waterfall, Doi Luang National Park, Muang District, Phayao Province, northern Thailand, 3.V.2005, by Wej Choochote. Paratypes: 3 males with their associated pupal exuviae and cocoons, 10 pupae, and 5 mature larvae, same data as holotype except date, 24. III. 2005.

**ECOLOGICAL NOTES.** The pupae and larvae of this new species were found attached to the surface of rocks in a fast flowing cascading stream (width 0.5–1.0 m, depth 10–20 cm, exposed to sun, water temperature 21–24°C, altitude 680 m) in a densely forested area. This species was found together with *Simulium (Simulium) mediocoloratum* Takaoka and Choochote.

**ETYMOLOGY.** The specific name *phayaoense* refers to the name of the province, Phayao, where this new species was collected.

**DISTRIBUTION.** Thailand.

**REMARKS.** *Simulium (Simulium) pha-*



*yaoense* sp. nov. is very similar in the female and male genitalia and pupal gills to *S. (S.) grossifilum* described from Peninsular Malaysia (Takaoka and Davies, 1995), and later reported from southern Thailand (Kuvangkadilok and Takaoka, 2000), but differs from the latter species by the following characters (those of *S. (S.) grossifilum* in parentheses): the female sensory vesicle 0.41 times as long as the third segment (0.34 times), the male abdominal segments 2, 6 and 7 each with a pair of whitish pruinose (silvery iridescent in light) spots (such paired spots present on segments 2, 5, 6 and 7), the last pupal abdominal segment lacking terminal hooks (with distinct terminal hooks), the larval postgenal cleft 2.4–2.8 times as long as the postgenal bridge (1.8–2.0 times), and the larval posterior circlet with 84–98 rows of hooklets (with ca. 120 rows of hooklets).

***Simulium (Simulium) pukaengense*  
sp. nov.**

**DESCRIPTION. Female.** Body length 3.0 mm. **Head.** Narrower than width of thorax. Frons brownish-black, shiny, with white pruinose (bluish iridescent at certain angle of light), and with several dark stout hairs along lateral margins; frontal ratio 1.6:1.0:1.3; frons-head ratio 1.0:3.1. Fronto-ocular area (Fig. 5A) well developed, triangular. Clypeus brownish-black, shiny, with marked bluish iridescent pruinosity, moderately covered with dark stout and slender hairs except medial portion of upper 1/2 bare. Labrum 0.65 times as long as clypeus. Antenna composed of 2+9 segments, dark brown to brownish-black except scape, pedicel, and base of 1st flagellar segment yellow. Maxillary palp brownish-black, with 5 segments, proportional lengths of 3rd, 4th, and 5th segments 1.0:1.1:2.4; 3rd segment (Fig. 5B) not enlarged; sensory vesicle ellipsoidal, with rugged surface, 0.42 times length of 3rd segment, with medium-sized round opening medially. Maxillary lacinia with 13–15 inner and 13 or 14 outer teeth. Mandible with 36–38 inner and 14 or 15 outer teeth. Cibarium (Fig. 5C) with prominent medial projection directed dorsally at its dorsal margin, and covered with numerous minute

spinous processes on medial projection and near base of projection. **Thorax.** Scutum brownish-black (except anterolateral calli ochreous or light brown), shiny, grey pruinose, with scutal patterns formed by pruinose and non-pruinose portions differing by the angles of light: i.e., when illuminated anterodorsally and viewed dorsally, 1 medial very narrow, 2 sub-medial rather wide, and 2 lateral rather wide longitudinal dark non-pruinose vittae, all connected to large dark non-pruinose portion on prescutellar area; color pattern reversed when illuminated dorsally and viewed posteriorly; scutum densely covered with yellow recumbent short hairs interspersed with dark long upright hairs on prescutellar area. Scutellum light brown, with dark long hairs as well as yellow short hairs. Postnotum brownish-black, shiny, and bare. Pleural membrane bare. Katepisternum longer than deep, shiny, and bare. **Legs.** Foreleg: coxa yellowish-white; trochanter light brown; femur yellowish on basal 1/2 and light to medium brown on apical 1/2; tibia largely yellowish-white except a little less than apical 1/3 brownish-black, with a large white sheen on outer surface when illuminated; tarsus brownish-black, with dorsal hair crest of moderate length; basitarsus dilated, 5.1 times as long as its greatest width. Midleg: coxa medium to dark brown except posterior surface brownish-black; trochanter light brown with basal 1/2 yellow; femur light to medium brown except basal 1/4 yellow; tibia yellowish-white except apical 2/5 brownish-black, with a large white sheen on posterior surface when illuminated; tarsus brownish-black except basal 1/5 of basitarsus dark yellow. Hind leg: coxa medium brown; trochanter yellow; femur light to medium brown with basal 2/5 yellowish-white to yellow and apical cap dark brown; tibia yellowish-white with a little more than apical 1/3 brownish-black, and with a large white sheen on posterior surface when illuminated; tarsus dark brown to brownish-black except a little more than basal 1/2 of basitarsus and basal 1/2 of 2nd tarsal segment yellowish-white; basitarsus (Fig. 5D) nearly parallel-sided, 5.8 times as long as wide, and 0.8 and 0.7 times as wide as greatest widths of hind tibia and femur, respectively; calcipala (Fig. 5D) short, nearly as long as its basal width and 0.37 times as wide as greatest width of basitarsus; pedisulcus (Fig. 5D) distinct at basal 1/3 of 2nd tarsal segment. Tarsal claws simple

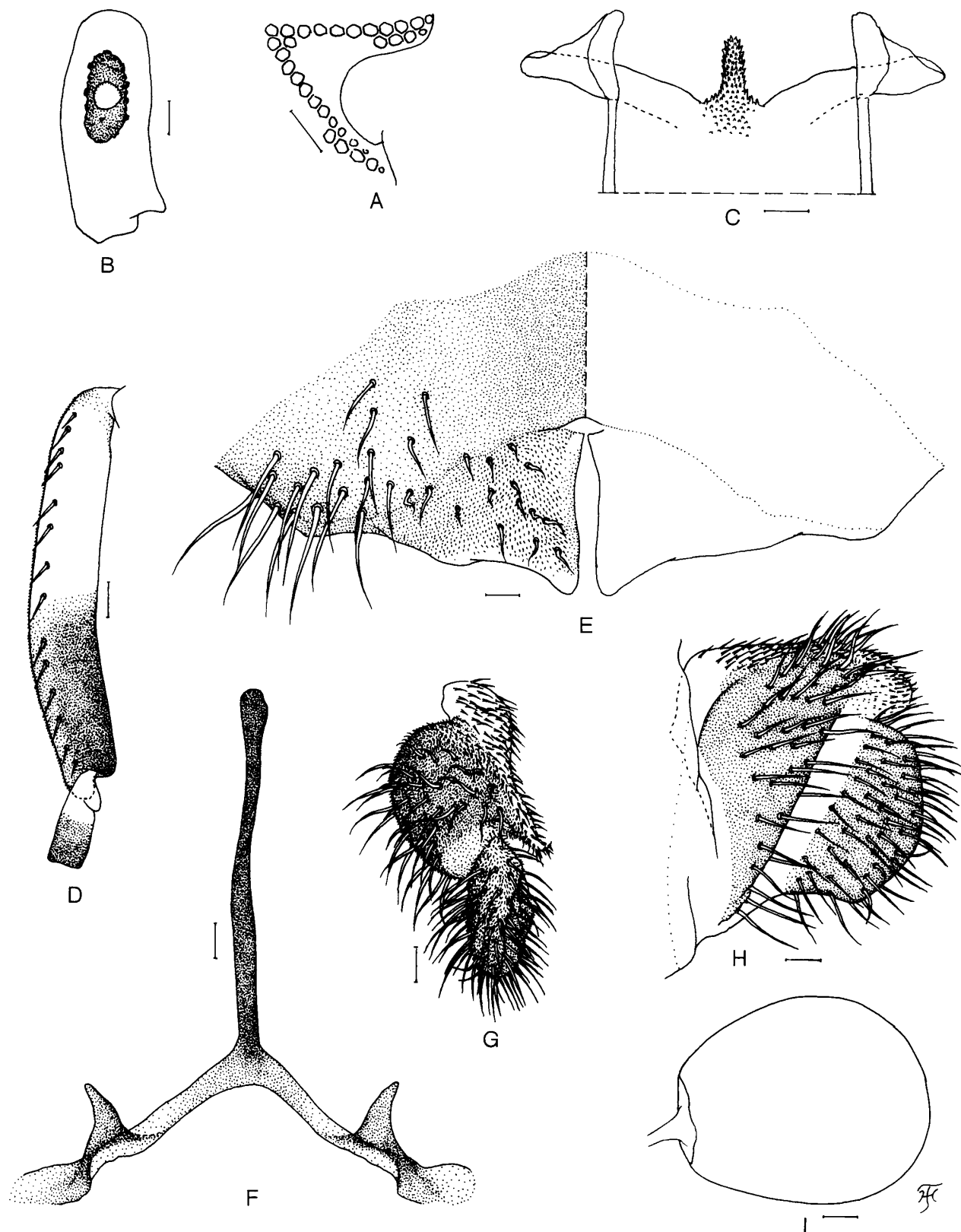


Fig. 5. Female of *Simulium* (*Simulium*) *pukaengense* sp. nov. A, fronto-ocular area (right side); B, 3rd segment of maxillary palp with sensory vesicle (right side, front view); C, cibarium; D, basitarsus and 2nd tarsal segment of hind leg showing calcipala and pedisulcus (left side, outer view); E, 8th sternite and ovipositor valves (ventral view); F, genital fork; G and H, paraprocts and cerci (right side; G, ventral view; H, outer view); I, spermatheca. Scale bars. 0.05 mm for A and D; 0.02 mm for B, C, and E-I.

without any tooth. **Wing.** Length 2.5 mm; costa with dark spinules and hairs; subcosta haired except apical 2/5 or 1/2 bare; basal section of vein R fully haired (though narrowly bare near base in 1 female);  $R_1$  with spinules and hairs;  $R_2$  with hairs only; hair tuft at base of stem vein dark brown; basal cell absent.

**Abdomen.** Basal scale ochereous, with fringe of dark hairs. Dorsal surface of abdomen greyish-brown except segment 2 almost entirely whitish-yellow, or whitish-yellow on anterior 1/2 and greyish on posterior 1/2, and with dark short hairs; tergites 2 and 6–9 shiny. Ventral surface of abdomen whitish-yellow to ochereous except segment 8 light to medium brown; segment 7 with a pair of small light brown submedial sternal plates. **Genitalia.** Sternite 8 (Fig. 5E) moderately sclerotized, with 21 or 22 long and medium-long stout hairs on each lateral surface. Ovipositor valves (Fig. 5E) nearly triangular, membranous, covered with 12 or 13 medium-long hairs as well as numerous microsetae except narrow areas along inner and posterior margins bare; inner margins weakly sclerotized, lightly darkened, concave medially, and somewhat separated from each other. Genital fork (Fig. 5F) of inverted-Y form, with narrow, well sclerotized stem; arms of moderate width, each with distinct projection directed anteriorly. Paraproct in ventral view (Fig. 5G) nearly as long as wide, with distinct concavity on anteroventral surface; paraproct in lateral view (Fig. 5H) about half as long as wide, moderately protruding ventrally beyond cercus forming round unpigmented apex, with numerous stout hairs on lateral and ventral surfaces. Cercus in lateral view (Fig. 5H) rounded posteriorly, about half as long as wide, with numerous stout hairs. Spermatheca (Fig. 5I) ovoid, well sclerotized (except portion of junction to duct unsclerotized), polygonal surface patterns faintly visible, and with internal setae; accessory ducts subequal in thickness to major duct.

**Male.** Body length 3.0–3.1 mm. **Head.** Slightly wider than thorax. Upper eye consisting of large facets in 20 horizontal and 19 vertical rows. Clypeus black, white pruinose, bluish iridescent when illuminated, covered with several dark long hairs along lateral margins (median portion bare). Antenna composed of 2 + 9 segments, dark brown except base of 1st flagellar segment pale; 1st flagellar segment elongate, 2.2 times as long as 2nd one. Maxil-

lary palp composed of 5 segments, proportional lengths of 3rd, 4th, and 5th segments 1.0 : 1.1 : 1.9; 3rd segment (Fig. 6A) of normal size, with medium-sized oblong sensory vesicle, 0.26 times length of 3rd segment, and with a small opening apically. **Thorax.** Scutum black, uniformly and densely covered with golden-yellow recumbent short hairs interspersed with dark short hairs near anterior margin and dark long upright hairs on prescutellar area; scutum with a large white pruinose shiny spot on each shoulder, extending posteriorly forming a broad white pruinose band along each lateral margin up to base of wing. Scutellum dark brown, with several dark long upright hairs as well as golden-yellow short hairs. Postnotum brownish-black, whitish-grey pruinose at certain angle of light, and bare. Pleural membrane bare. Katepisternum longer than deep, and bare. **Legs.** Foreleg: coxa yellow; trochanter medium brown except base yellow; femur medium to dark brown; tibia dark brown to brownish-black except medial wide portion along outer margin narrowly yellowish white, and with a large white sheen on outer surface of basal 3/5 when illuminated; tarsus brownish-black, with dorsal hair crest of moderate length; basitarsus somewhat dilated, 5.7 times as long as its greatest width. Midleg: coxa brownish-black; trochanter and femur dark brown with apical cap of femur brownish-black; tibia dark brown to brownish-black except basal 2/5 yellow and with a whitish-yellow sheen on posterior surface of basal 3/4 when illuminated; tarsus brownish-black. Hind leg: coxa dark brown; trochanter yellow; femur dark brown except base yellowish-white or yellow and apical cap brownish-black; tibia dark brown to brownish-black except extreme base yellow; tarsus medium to dark brown (basal 1/2 of 2nd segment somewhat lighter in some males); basitarsus (Fig. 6B) somewhat enlarged, spindle-shaped, 4.1–4.4 times as wide as its greatest width at apical 1/3, and 0.8–0.9 times as wide as greatest width of hind tibia, which is nearly as wide as that of hind femur; calcipala (Fig. 6B) small, 0.8 times as long as wide and 0.3 times as wide as greatest width of hind basitarsus; pedisulcus (Fig. 6B) distinct at basal 2/5 of 2nd tarsal segment. **Wing.** Length 2.2–2.3 mm; other characters including fully-haired basal portion of radial vein as in female except subcosta completely bare. **Abdomen.** Basal scale brownish-black, with fringe of dark

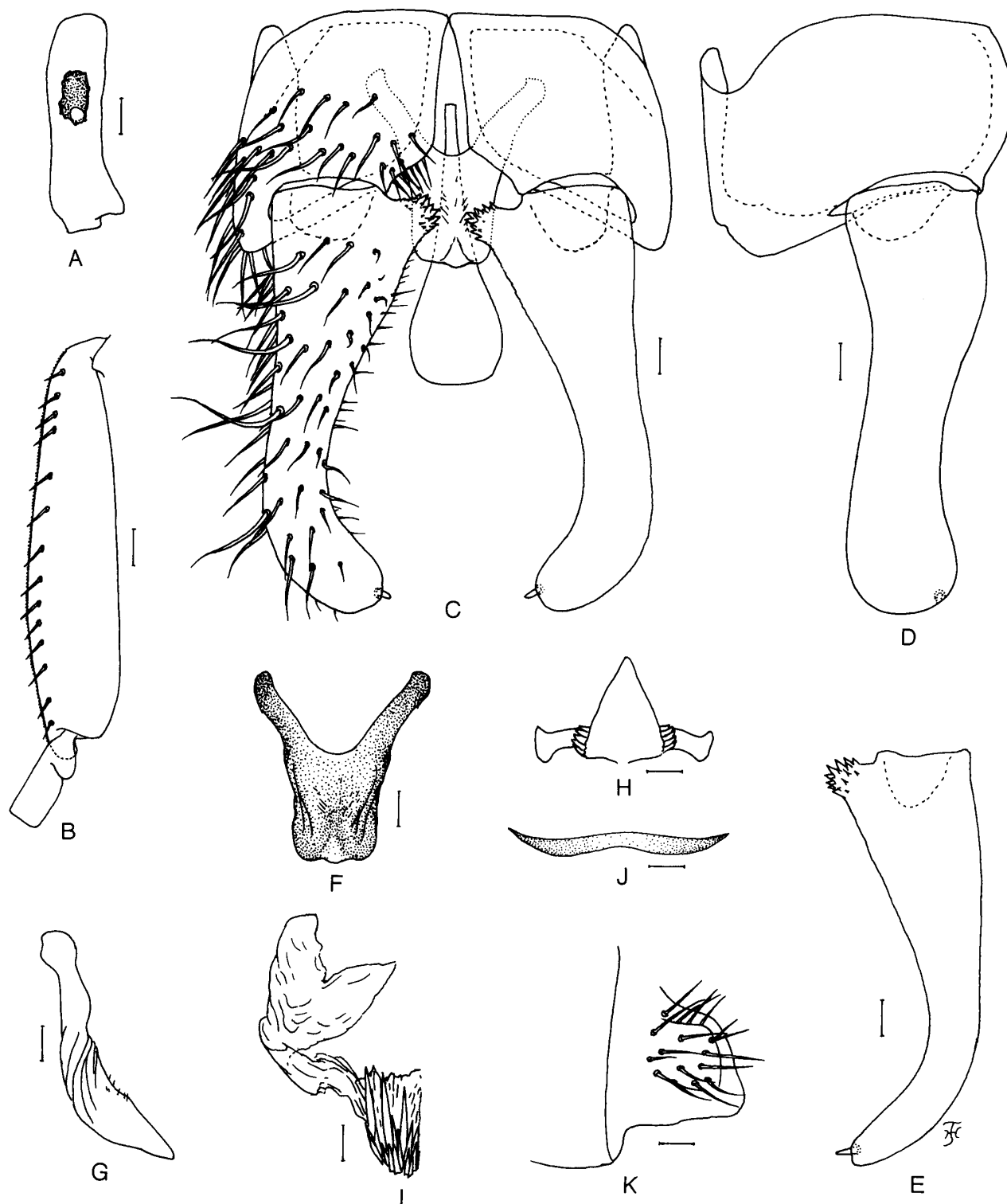


Fig. 6. Male of *Simulium* (*Simulium*) *pukaengense* sp. nov. A, 3rd segment of maxillary palp with sensory vesicle (right side, front view); B, basitarsus and second tarsal segment of hind leg showing calcipala and pedisulcus (left side, outer view); C, coxites, styles, ventral plate and median sclerite *in situ* (ventral view); D, coxite and style (right side, ventrolateral view); E, style showing basal protuberance (left side, medial view); F-H, ventral plates (F, ventral view; G, lateral view; H, end view); I, paramere (left side, dorsal view); J, dorsal plate (ventral view); K, 10th abdominal segment and cercus (right side, lateral view). Scale bars. 0.05 mm for B; 0.02 mm for A, and C-K.

long hairs. Dorsal surface of abdomen brownish-black except basal 1/2 of 2nd segment ochreous or light brown, with dark short hairs; tergites 2, 6 and 7 each with a pair of white pruinose spots (bluish iridescent when illuminated); those on tergite 2 situated dorso-laterally and connected to each other, those on tergites 6 and 7 situated dorsolaterally and laterally, respectively. **Genitalia.** Coxite in ventral view (Fig. 6C) nearly quadrate, and in ventrolateral view (Fig. 6D) much wider than long. Style in ventral view (Fig. 6C) elongate, much longer than coxite, gradually narrowed from base to middle, then nearly parallel-sided or slightly widened apically, with apex rounded and directed inwardly, and with a small terminal spine; style in ventrolateral view (Fig. 6D) wide along basal 1/3 (ca. 0.34 times length of style), then narrowed medially (narrowest width ca. 0.6 times as wide as greatest width near base) and somewhat widened apically; style in medial view (Fig. 6E) gradually narrowed from base to a little more than basal 1/2, then nearly parallel-sided toward apex, and with distinct basal protuberance bearing several stout conical spines at and near apex. Ventral plate in ventral view (Fig. 6C, F) with body heavily sclerotized, rectangular, longer than wide, nearly parallel-sided, with a few longitudinal ridges on each side, very sparsely covered with setae on ventral surface (ca. 12 setae), and with well sclerotized arms diverged from each other; ventral plate in lateral view (Fig. 6G) with a few ridges running horizontally on its body, and with a prominent bare process directed ventroposteriorly; ventral plate in end view (Fig. 6H) with body bearing bare triangular process directed ventrally. Median sclerite in ventral view (Fig. 6C) well sclerotized, long, plate-like, narrow basally, gradually widened toward apex, and with round apex. Paramere (Fig. 6I) wide basally, with numerous parameral hooks. Aedeagal membrane densely covered with needle-like setae, with moderately sclerotized narrow dorsal plate (Fig. 6J). Segment 10 with or without 1 distinct hair near base of each cercus. Cercus (Fig. 6K) rounded with 17 or 18 distinct hairs.

**Pupa.** Body length (excluding gill filaments) 2.5–3.0 mm. **Head.** Integument yellowish-brown, moderately covered with tubercles of various sizes, large tubercles (Fig. 7A) mostly located on frons and with a few to several

minute secondary projections peripherily, and smaller conical or round tubercles without secondary projection on face; frons with 2 pairs of very long fan-like trichomes situated close together, dorsal one with 10–13 long branches, and ventral one with 18–20 long branches (Fig. 7B); face with a pair of very long trichomes each with 12–14 long branches (Fig. 7C); antennal sheath with 9 moderately raised portions corresponding to flagellar segments 1–9, and moderately covered with smaller round tubercles. **Thorax.** Integument yellowish-brown except posterior 1/2 yellowish, moderately covered with large tubercles (similar to those on frons) dorsally and laterally on anterior 1/2 and smaller ones dorsally and laterally on posterior 1/2; thorax anteriorly with 3 dorsomedial very long trichomes (2 with about 13 long branches, 1 with about 25 long branches) (similar to those on frons), and 2 dorsolateral pairs of very long trichomes each with 16–18 long branches (similar to those on frons), posteriorly with 1 lateral pair of long trichomes with 5–7 long branches (Fig. 7D), and ventrolaterally with 3 pairs of trichomes (1 medium-long and with 2–4 branches, 1 very long and with 4–7 long branches, 1 very long and bifid or trifid) (Fig. 7E, F, G). Gill (Fig. 7H) with 6 short thread-like filaments arranged in 3 pairs (dorsal pair short-stalked, and middle and ventral pairs sessile); 2 filaments of dorsal pair subequal in thickness to each other, and thickest of all, followed by inner filament of middle pair which is slightly thicker than its outer counter filament, and 2 filaments of ventral pair thinnest of all, about 0.6 times as thick as filaments of dorsal pair when compared near base; 2 filaments of dorsal pair longest of all (1.5–1.9 mm), followed by those of middle pair, and 2 filaments of ventral pair shortest (1.0–1.2 mm); all filaments very slightly to moderately pigmented and light yellowish-white to yellowish-brown (2 filaments of dorsal pair usually darker than others), gradually tapered from base to apex, with annular ridges and furrows throughout their length, and densely covered with minute tubercles. **Abdomen.** Dorsally, segment 1 light brown, bare or very sparsely covered with minute tubercles, and with 1 simple (or bifid) medium-long hair-like seta (Fig. 7I) on each side; segment 2 almost transparent, with 1 simple medium-long hair-like seta and 5 short spinous setae on each side; segments 3 and 4 transparent, each with 4

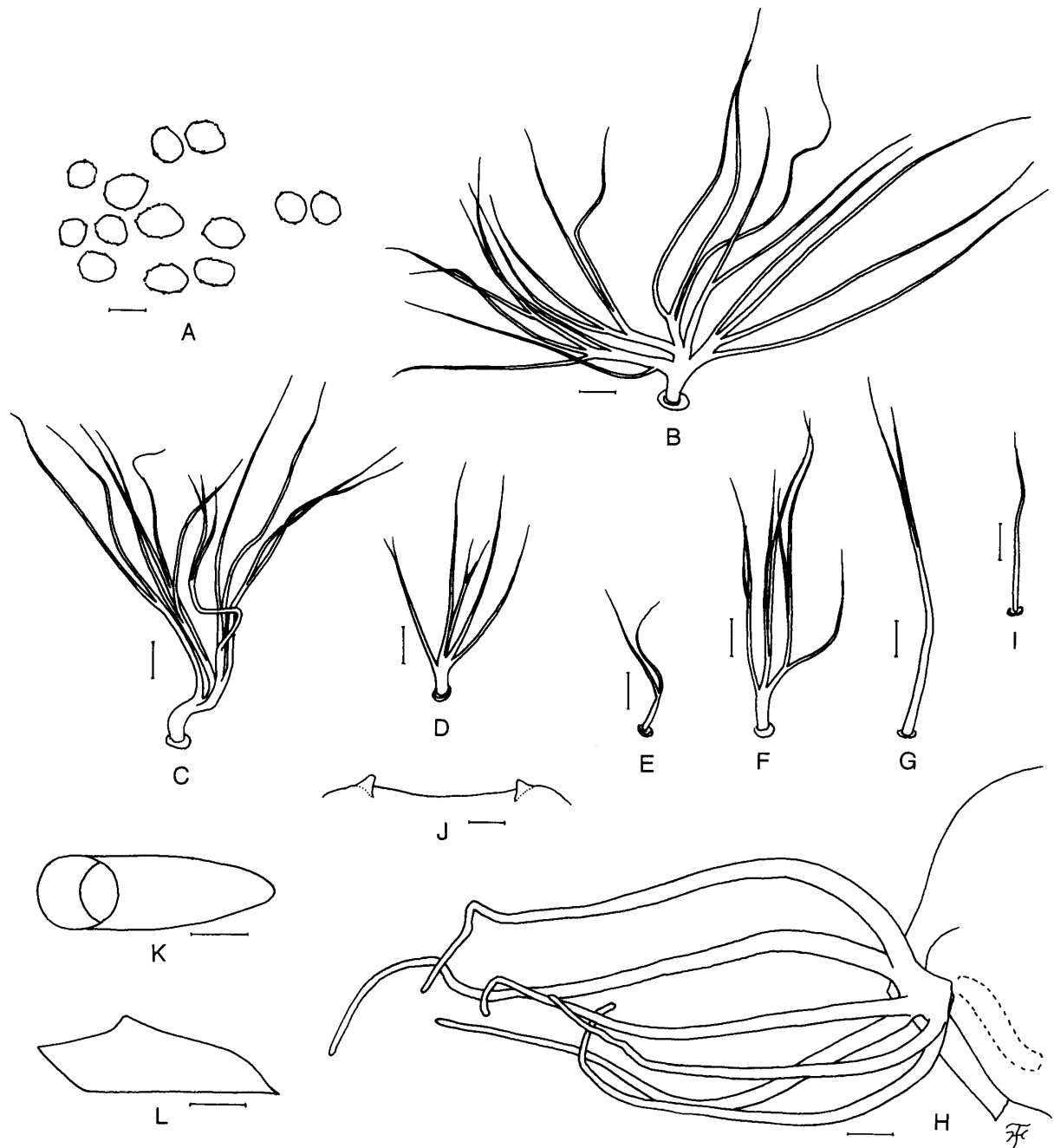


Fig. 7. Pupa of *Simulium* (*Simulium*) *pukaengense* sp. nov. A, tubercles on frons; B, ventral one of 2 frontal trichomes; C, facial trichome; D-G, thoracic trichomes (D, posterolateral; E-G, ventrolateral); H, gill filaments (left side, outer view); I, hair-like seta on dorsal surface of 1st abdominal segment; J, terminal hooks (dorsal view); K and L, cocoons (K, dorsal view; L, lateral view). Scale bars. 1.0 mm for K and L; 0.1 mm for H; 0.02 mm for B-G, I and J; 0.01 mm for A.

hooked spines along posterior margin on each side; segments 5 and 6 transparent, without spine-combs; segments 7 and 8 transparent, each with a transverse row of spine-combs on each side (though spine-combs on segment 7 usually smaller in size and fewer in number than those on segment 8); segment 9 weakly sclerotized and yellowish, sparsely covered

with minute tubercles, with or without spine-combs (which are, if present, subequal in size to, or smaller than, those on segment 7), and with a pair of small conical terminal hooks (Fig. 7J); segments 6-9 each with a transverse row of comb-like groups of minute spines on each side. Ventrally, segment 4 with 1 simple slender short hair-like hooklet (much smaller

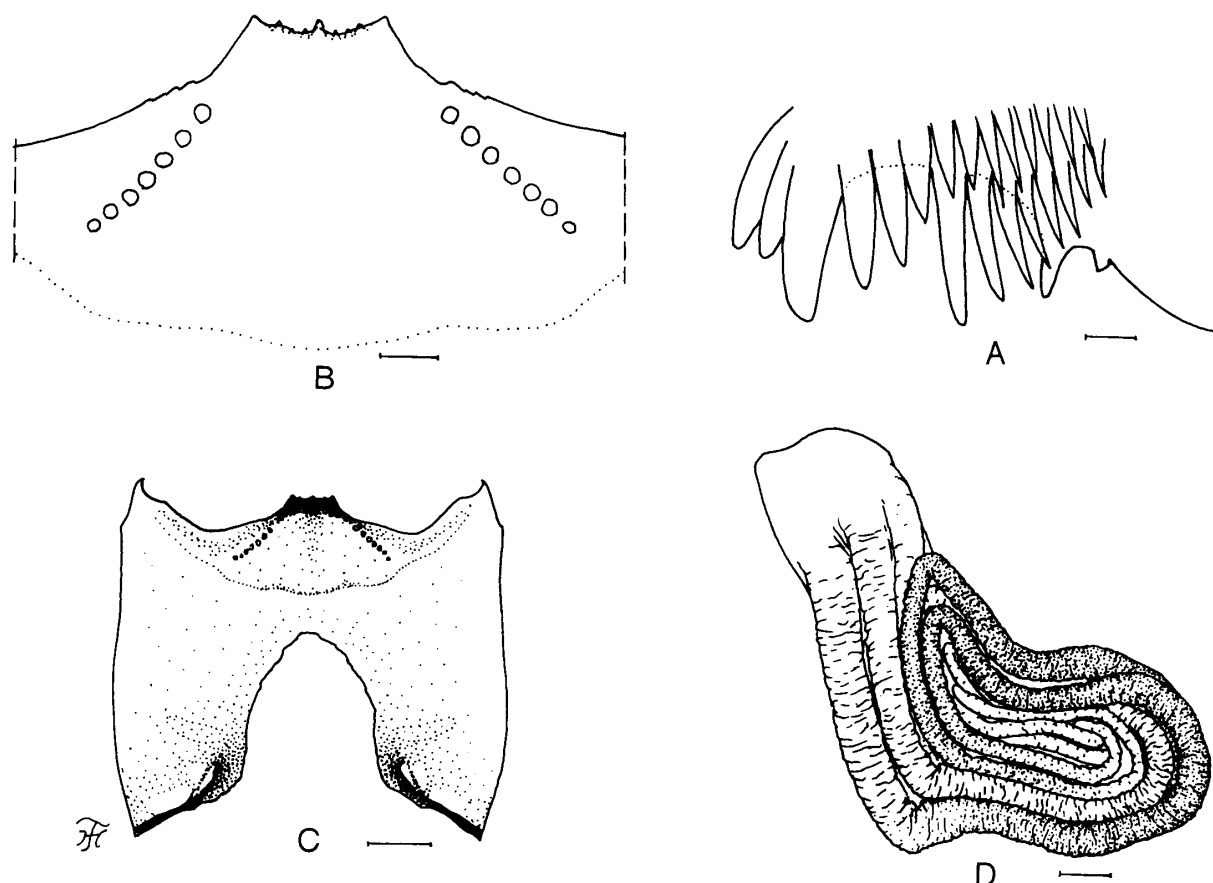


Fig. 8. Mature larva of *Simulium* (*Simulium*) *pukaengense* sp. nov. A, apical portion of mandible; B, hypostomium; C, head capsule showing postgenal cleft (ventral view); D, pharate pupal gill (left side, outer view). Scale bars. 0.1 mm for C; 0.05 mm for D; 0.03 mm for B; 0.01 mm for A.

than hooks on segments 5–7) or simple short hook (somewhat smaller than those on segments 5–7) and a few short setae on each side; segments 5–7 each with a pair of bifid inner and simple outer hooks on each side; segments 4–8 each with comb-like groups of minute spines. Grapnel-like hooklets absent. **Cocoon** (Fig. 7K, L). Shoe-shaped, yellowish white, thin, without any open spaces or windows, and not extending ventrolaterally; 3.8–4.2 mm long by 1.2–1.4 mm wide.

**Mature larva.** Body length 6.0–6.6 mm. Body color usually light greyish-green. Abdomen gradually widened from 1st segment toward segment 7, then narrowed to posterior tip when viewed laterally. Cephalic apotome pale yellow to yellow except small dark area in middle along posterior border; posteromedial longitudinal spot usually faintly positive, and mediolateral spot on each side also usually faintly positive or merged into ground color or at times faintly negative, other spots usually indistinct, merged into ground color; lateral and ventral surfaces of head capsule variable

in color by individual larvae: lateral surface from nearly yellowish to light brown except eye-spot region whitish; all spots near posterior margin positive or indistinct, all or some spots ventral to eye-spot region positive or indistinct or negative; ventral surface somewhat dark yellow (though usually darkened along margin of postgenal cleft) to medium brown; horizontal long spot on each side of postgenal cleft positive, indistinct or negative. Antenna composed of 3 segments and apical sensillum, somewhat longer than stem of labral fan; length ratio of segments (from base to tip) 1.0 : 1.6 : 0.6. Labral fan with 43–46 main rays. Mandible (Fig. 8A) with 2 usual mandibular serrations (1 medium-sized, 1 small); main tooth at an obtuse angle to mandible on apical side; supernumerary serrations absent; comb-teeth decreased in length from 1st to 3rd, or 1st and 2nd teeth subequal in length to each other, and longer than 3rd one. Hypostomium (Fig. 8B) with 9 apical teeth, of which median tooth and each corner tooth subequal in length to each other, and longer than others; lateral

margins weakly serrate apically; 7 hypostomal bristles widely divergent posteriorly from lateral border on each side. Postgenal cleft (Fig. 8C) deep, bullet-shaped, 3.1–4.0 times as long as postgenal bridge. Cervical sclerites composed of 1 narrow stick-like and 1 elliptical pieces usually not fused to each other on each side, not fused to occiput, moderately separated medially from each other. Thoracic cuticle almost bare. Pharate pupal gill (Fig. 8D) with six thread-like filaments, of which 2 filaments of dorsal pair darkened (though basal and apical portions pale), and 4 other filaments nearly pale. Abdominal cuticle bare except last segment moderately covered with short colorless setae on each side of anal sclerite. Rectal scales not discernible. Rectal organ compound, each of 3 lobes with 19–28 finger-like secondary lobules. Anal sclerite X-shaped, with broadened anterior arms about 0.7 times as long as posterior ones; basal juncture area with narrow deep incision opening posteriorly, and without sensilla on it; 4–6 sensilla just posterior to posterior arms. Ventral papillae absent. Posterior circlet with 144–164 rows of hooklets with up to 22–24 hooklets per row.

**TYPE SPECIMENS.** Holotype female with associated pupal exuvia and cocoon, reared from a pupa collected from a cascading stream, Pu Kaeng Waterfall, Doi Luang National Park, Chiang Rai Province, northern Thailand, 3. V. 2005, by W. Choochote. Paratypes: 1 female, 5 males, all adults with associated pupal exuviae and cocoons, 7 pupae, and 15 mature larvae, same data as holotype.

**ECOLOGICAL NOTES.** The pupae and larvae of *S. (S.) pukaengense* were found attached to the surface of the stream-bed rock in a fast-flowing cascading stream (width 0.5 m, depth 10–15 cm, exposed to sun, water temperature 23°C, altitude 640 m) in a sparsely forested area. This new species was collected together with *Simulium (Simulium) mediocoloratum* Takaoka and Choochote, and *S. (S.)* sp. belonging to the *multistriatum* species-group.

**ETYMOLOGY.** The specific name *pu-*

*kaengense* refers to the name of the waterfall, Pu Kaeng, where this species was collected.

**DISTRIBUTION.** Thailand.

**REMARKS.** *Simulium (S.) pukaengense* sp. nov. appears to be closely related to *S. (S.) digrammicum* Edwards reported from Peninsular Malaysia and northern Thailand (Edwards, 1928; Takaoka and Davies, 1995; Takaoka and Choochote, 2002) because both species share many characters including two rare characters as follows: the basal portion of the radial vein of both sexes of adults fully haired (usually bare in the male), and the male abdominal segments 2, 6 and 7 each with a pair of white or whitish-grey pruinose dorsolateral or lateral spots (usually such paired spots present also on the segment 5). However, *S. (S.) pukaengense* is distinguished from the latter known species by the following characters (those of *S. (S.) digrammicum* in parentheses): the cibarium bearing minute spinous processes near the base of the medial projection as well as on the medial projection itself (Fig. 5C) (only on the medial projection) in the female; body of the ventral plate nearly parallel-sided (Fig. 6F) (gradually narrowed toward the posterior tip), and hind femur subequal to hind tibia and slightly wider than hind basitarsus (hind femur slightly narrower than both hind tibia and basitarsus) in the male; cocoon without any lateral window (Fig. 7L) (with a lateral window on each side) and the middle pair of gill filaments sessile (Fig. 7H) (short-stalked) and dorsal spinecombs always present on the abdominal segments 7 and 8 (present only on the abdominal segment 8) in the pupa; first comb-tooth as long as, or slightly longer than, the second one (Fig. 8A) (first comb-tooth apparently shorter than the second one), and rectal organ with 19–28 secondary lobules per lobe (with 14–17 secondary lobules per lobe) in the mature larva.



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